ABSTRACT

The present invention is to provide a multiple detection method that can detect contaminating microorganisms existing in foods, including pathogenic Escherichia coli O157, Listeria monocytogenes and Salmonella spp., with high sensitivity comparable or even superior to official methods, comprising the steps of amplifying a plural number of target genes with a single PCR reaction tube and analyzing the same. The following steps are performed consecutively: (A) a step of extracting DNA of the target microorganisms to be detected by treating with at least a lytic enzyme such as Achromopepidase and Lyzocyme and/or bacteriocin having lytic activity such as Enterolysine, a surfactant and a protein denaturing agent; and (B) a step of mixing a specific primer to the target microorganisms to be detected to perform multiplex PCR. Further, it is preferable to add a step of culturing with a culture condition where 1 CFU/100 g microorganisms becomes 10^3 CFU/ml or more after 18 to 48 h of culture, for example that the pH after culture becomes 5.1 or more, before the step of extracting DNA of the target microorganisms to be detected.